

Antipsychotic Drugs Influence Dopamine Neuron Terminals via Action on D2-receptors and Vesicles

Alex Klausing

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College of Pharmacy
The Ohio State University

Advisor: Lane J. Wallace

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Abstract

Dopamine D₂ antagonist antipsychotic drugs share the effects of increasing dopamine synthesis, dopamine neuron firing rate, and dopamine and DOPAC levels. These antagonists all raise extracellular dopamine to approximately 163% of control and DOPAC levels to approximately 200% of control without changing tissue level of dopamine. Variability in striatal tissue DOPAC level (190 – 350% of control) and rates of dopamine synthesis (190-378% of control) reported after administration of various antipsychotic drugs gives evidence that these parameters are influenced by factors besides the antagonism of the D₂ receptor. We used a computational model of dopaminergic terminals in the striatum to determine what parameters other than D₂ receptors might be targets for the drugs. Simulation model results suggest that D₂ receptor antagonism results in a slight increase in rate of dopamine exocytosis accompanied by a small increase in rate of dopamine synthesis needed to maintain the increase in rate of exocytosis and a small increase in rate of dopamine synthesis specifically dedicated to DOPAC secretion. Simulation model results also suggest that variable increases in levels of tissue DOPAC result from increases in passive diffusion of dopamine out from vesicles coupled with an increase in rate of dopamine synthesis required to maintain tissue levels of dopamine. Biochemical data reported in the literature suggests changes in passive diffusion depend on the ability of the antipsychotic drug to diffuse into and alkalinize vesicles. This variable increase in tissue DOPAC, but invariable increase in extracellular dopamine across all antipsychotic drugs, provides evidence that DOPAC levels are not a precise indicator of dopamine turnover. These findings also suggest there is a sensor that monitors dopamine vesicular levels and provides feedback to tyrosine hydroxylase activity.

Introduction

All dopamine D₂ receptor antagonist drugs have efficacy in treating schizophrenia, with affinity for interacting with dopamine D₂ receptors correlating with clinical potency (Seeman, 1980). The D₂ family consists of D₂, D₃, and D₄ receptors (Sokoloff and Schwartz, 1995). D₂ family receptors are found on dendrites and soma of dopaminergic neurons, on dopaminergic axon terminals, and at post-synaptic sites on several different kinds of neurons (Hersch et al., 1995; Roth, 1984). The receptors found on dendrites and cell bodies in the substantia nigra and ventral tegmental area control firing rate of dopaminergic neurons, with activation of these receptors decreasing firing rate (Bunney et al., 1973). The D₂ autoreceptors found on dopamine terminals are thought to regulate tyrosine hydroxylase activity (Dunkley et al., 2004; Lindgren et al., 2003; Lindgren et al., 2001; Nagatsu et al., 1964) and quantal size (Benoit-Marand et al., 2001).

Drugs that are antagonists at dopamine D₂ receptors impact the amount and subcellular distribution of dopamine and its major metabolite, DOPAC (see Fig 2). All D₂ antagonists increase striatal DOPAC content. DOPAC level was used as an index of dopamine turnover before the advent of the microdialysis technique. Dopamine in cytosolic location is metabolized by monoamine oxidase and aldehyde dehydrogenase to produce DOPAC. Thus, changes in level of DOPAC are thought to reflect changes in level of cytosolic dopamine. A major portion of dopamine in the cytosol arrives there through a recycling process. Dopamine is released from vesicles into the extracellular space during a signaling event and then recaptured into the cytosol via the dopamine transporter. This dopamine remains in the cytosol for a short time until it is transported into vesicles, where it is protected from metabolism. Thus, changes in cytosolic dopamine are thought to parallel changes in dopamine in extracellular signaling compartment. Based on this concept, the hypothesis was developed that antipsychotic drugs increase dopamine turnover.

With the advent of the microdialysis technique (Zetterstrom et al., 1983), extracellular levels of dopamine could be directly measured. Such studies showed that dopamine D₂ antagonists elicit a small increase in extracellular dopamine, implying a small increase on dopamine turnover (see Fig 2). Dialysis-based estimates of dopamine turnover elicited by dopamine D₂ antagonist drugs are smaller than estimates based on tissue levels of DOPAC (see Fig 2). In addition, recent modeling studies show that an alternative source for tissue dopamine is passive diffusion of the neurotransmitter out of vesicles (Wallace, 2007). Further complicating the picture, dialysis studies show that a significant amount of DOPAC is found in extracellular compartment (see Fig 2). Thus, there are several unanswered questions relative to the impact of antagonists at dopamine D₂ receptors on dopamine and DOPAC levels and subcellular distributions.

The goal of this work is to explain the effects of antipsychotic drugs on striatal dopamine metabolism, including providing a quantitative estimate of dopamine and DOPAC levels in compartments within the varicosity. Our tool for doing this study is a computer model of striatal dopamine varicosities that has successfully explained changes in dopamine metabolism following inhibition of dopamine neuron firing rate (Wallace, 2007), certain actions of amphetamine (Wallace, 2012; Wallace and Connell, 2008), and the observation that specific activity of DOPAC is higher than specific activity of dopamine following injection of radioactive tyrosine precursor (Wallace and Traeger, 2012). As we proceeded with the initial phases of this work, it became apparent that the effects of antipsychotic drugs on DOPAC could not be explained with a model having only one source of DOPAC production. This, along with published data from a variety of experiments, led us to use a model involving a DOPAC synthesis-secretory complex. This proposed complex utilizes a pool of dopamine that is processed to DOPAC and immediately secreted to extracellular compartment. Our results suggest that all dopamine D₂ antagonist drugs increase extracellular dopamine through an increase in rate of exocytosis. All dopamine D₂ antagonist drugs stimulate the DOPAC synthesis- secretory complex, resulting in an increase in extracellular DOPAC and a small increase in total tissue DOPAC. In addition, the dopamine D₂ antagonist drugs appear to alkalinize storage vesicles to varying extent. This results in an increase in passive diffusion of dopamine out from vesicles where the neurotransmitter is susceptible to the action of monoamine oxidase, providing a source for increased tissue DOPAC.

Methods

Data from literature

For extracellular dopamine and DOPAC, studies showing effects of drugs in dialysis studies were analyzed. Levels of extracellular dopamine and DOPAC as a function of time were graphed. These plots showed that effects of all drugs analyzed are long lasting. Analyte levels increase over the first couple of sampling periods (20-40 min) and then remain at a plateau level for at least an hour. The mean value of data associated with sampling points between 60 and 120 minutes was calculated for each curve. The mean values were then used to construct dose-response curves, and the maximum response was used (except for remoxipride for which data for only one dose was found and this was assumed to be maximum response). Dose response curves were also constructed for tissue levels DOPAC and for rates of dopamine synthesis. For these parameters, the dose-response graphs did not show a clear maximum response. For a target value for our modeling output for these parameters, we chose the drug effect to be the values associated with the highest dose for which data are available for extracellular dopamine and DOPAC.

Model and Simulations

A computational model (Fig 1) of a striatal dopaminergic varicosity was used in order to simulate the antipsychotic data. In the model, dopamine is distributed between vesicle, cytosol, extracellular, and DOPAC synthesis-secretory compartment. DOPAC is distributed between cytosol extracellular and DOPAC synthesis-secretory compartment. Dopamine synthesizing and metabolizing enzymes are located in both the cytosol and the DOPAC synthesis-secretory compartment. A mechanism for DOPAC disappearance is located in cytosol and extracellular compartments. Transporters are included to move dopamine from extracellular to cytosol and from cytosol to vesicles. Details of the model are described extensively in Wallace, 2012, and Wallace and Traeger, 2012.

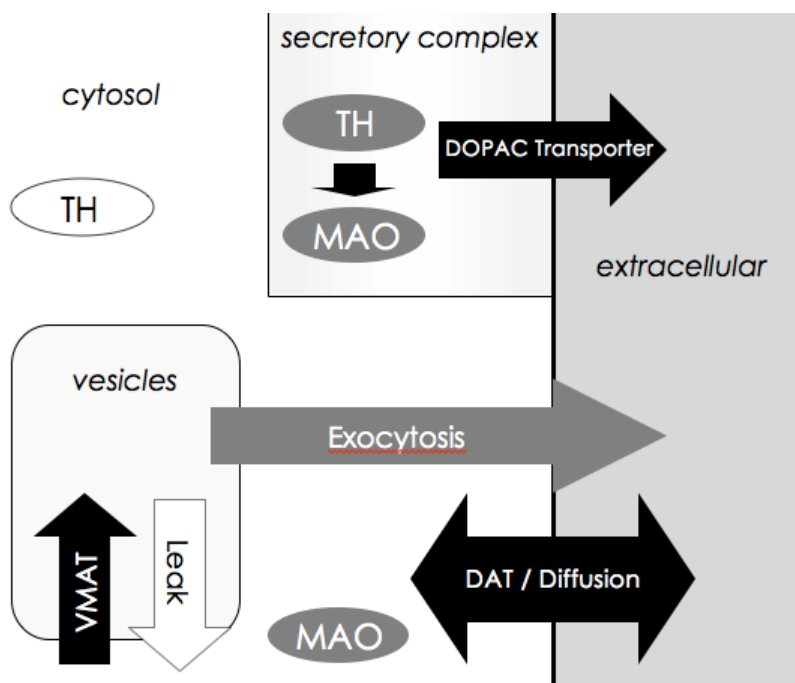


Fig 1. Schematic Diagram of Computational Model

Enzymatic processes are displayed in ovals. Arrows represent transporters or passive diffusion. Black shading indicates processes not affected by D₂ antagonist. Gray shading indicates processes affected by all D₂ antagonists to similar extent. White shading indicates processes affected by all D₂ antagonists with variable effects. Abbreviations: DAT = dopamine transporter, MAO = monoamine oxidase, TH = tyrosine hydroxylase, VMAT = vesicular monoamine transporter.

A simulation is initiated by assigning rate constants and maximum values for enzymes and transmitters and inputting initial values for dopamine and DOPAC in each compartment. Each program iteration represents a time step. At each program iteration, the amount of dopamine and DOPAC in each compartment is recalculated by adding or subtracting to the previous level of compound the amount added or removed from compartments by synthesis, metabolism, transport, and diffusion. Five minutes of baseline conditions are obtained; then alterations in rate constants for enzymes and transmitters associated with the drug being evaluated are input into the program. Simulations typically modeled the effects of 120 minutes of drug action.

Results

Our literature review showed that none of the five antipsychotic drugs evaluated change tissue dopamine levels (Fig 2a). All of the antipsychotic drugs evaluated produce nearly the same maximum increase in extracellular dopamine, with an average of 163% (Fig 2b). All of the antipsychotic drugs evaluated produce nearly the same maximum increase in extracellular DOPAC, with an average of 200% (Fig 2c). The increase in tissue DOPAC elicited by the antipsychotic drugs evaluated varied substantially (Fig 2d) as did the increase in *in vivo* rates of dopamine synthesis (Fig 2e). The amounts of increase in tissue DOPAC and of rate of *in vivo* dopamine synthesis were highly correlated. We assume that all drugs sharing the property of being antagonists at dopamine D₂ receptors produce equal magnitude of response resulting from this action. This suggests that the increases in extracellular dopamine and DOPAC result from block of dopamine D₂ receptors and that the increases in total tissue DOPAC and in rate of dopamine synthesis involve some mechanism in addition to block of dopamine D₂ receptors.

Fig 2. Effects of Dopamine D₂ Receptor Antagonist Drugs on Dopamine and DOPAC in Striatum.



Fig 2a. Antipsychotic drugs do not change dopamine tissue levels. Data derived from (Bjork et al., 1994; Boyar and Altar, 1987; Fowler et al., 1987; Fujiwara, 1992; Kuballa et al., 2005; Kuczenski, 1980; Magnusson et al., 1987b; Merchant et al., 1996; Ogren et al., 1986; Ravina et al., 2000; Schoemaker et al., 1997; Shore, 1976; Soares-da-silva, 1987; Waldmeier et al., 1985)

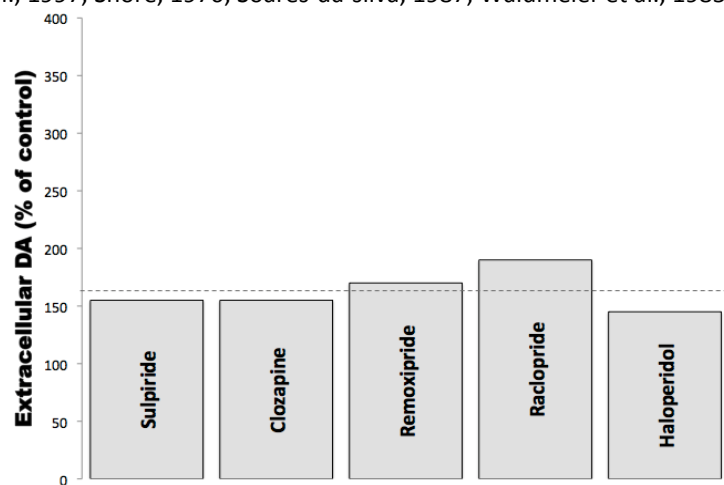


Fig 2b. Antipsychotic drugs elicit a modest rise in extracellular dopamine levels with an average of 63% increase from control. Data derived from (Di Chiara and Imperato, 1985; Gobert et al., 1995;

Gray and Connick, 1998; Gudelsky et al., 1992; Guinetdinov et al., 1994; Ichikawa and Meltzer, 1991; Imperato and Dichiaro, 1985; Meltzer et al., 1994; Millan et al., 1998; Moghaddam and Bunney, 1990; Rayevsky et al., 1995; See, 1991; See et al., 1991; Waters et al., 1993; Westerink et al., 1987b; Westerink and Vries, 1989; Zetterstrom et al., 1985; Zetterstrom et al., 1986)

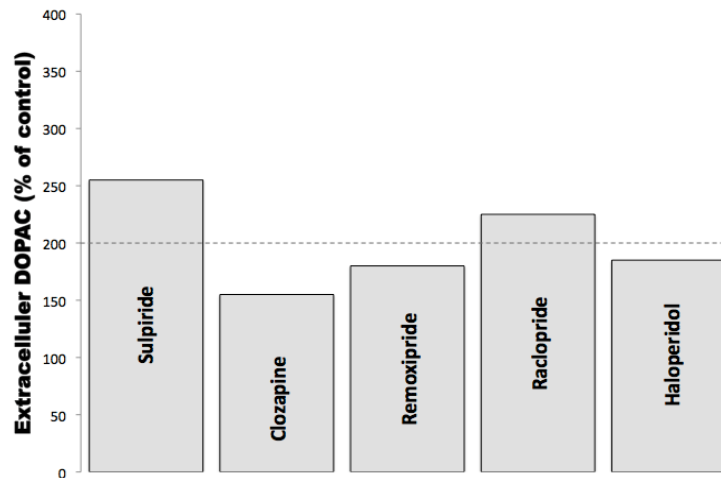


Fig 2c. All drugs elicit a modest rise in extracellular DOPAC with an average of 100% increase from control. Data derived from (Di Chiara and Imperato, 1985; Gudelsky et al., 1992; Guinetdinov et al., 1994; Ichikawa and Meltzer, 1991; Imperato and Dichiaro, 1985; Maidment and Marsden, 1987; Meltzer et al., 1994; Millan et al., 1998; Rayevsky et al., 1995; Rivest et al., 1991; Rivest and Marsden, 1992; See, 1991; See et al., 1991; Waters et al., 1993; Westerink and Vries, 1989; Zetterstrom et al., 1985; Zetterstrom et al., 1986)

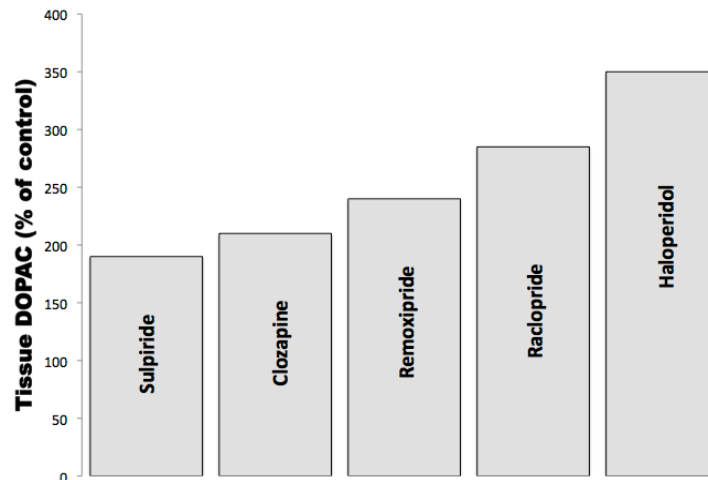


Fig 2d. The amount of increase in tissue DOPAC levels varies between the different drugs. Data derived from (Batool et al., 2010; Bjork et al., 1994; Boyar and Altar, 1987; Brougham et al., 1991; Fekete et al., 1978; Fowler et al., 1987; Fujiwara, 1992; Gudelsky et al., 1992; Hofmann et al., 1979a; Hofmann et al., 1979b; Imazu et al., 1989; Karoum and Egan, 1992; Kuballa et al., 2005; Kuczenski, 1980; Magnusson et al., 1987b; McMillen, 1981; Merchant et al., 1996; Moriuchi et al., 1995; Ogren et al., 1984; Ravina et al., 2000; Saller and Salama, 1986; Scatton, 1981; Scatton et al., 1977; Schoemaker et al., 1997; Shore, 1976; Soares-da-silva, 1987; Sorensen et al., 1993; Stanley and Wilk, 1979; Waldmeier et al., 1985; Westerink, 1979; Westerink et al., 1987a; Wilk et al., 1975; Wood et al., 1983)

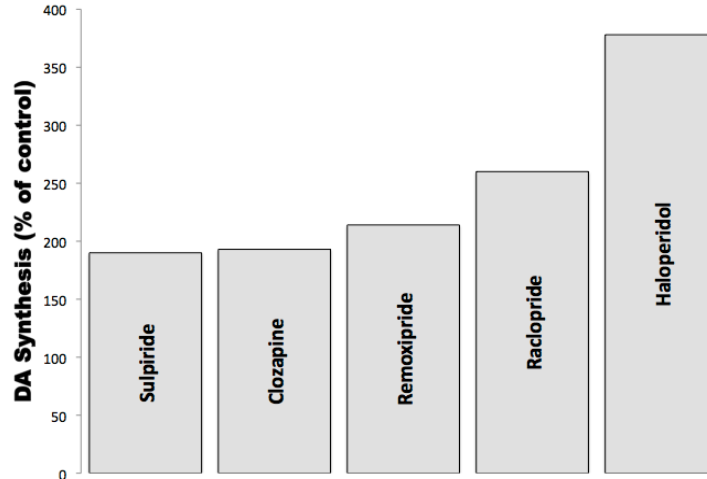


Fig 2e. The amount of increase in rate of dopamine synthesis varies between the different drugs. Data derived from (Alander et al., 1980; Argiolas et al., 1982; Audinot et al., 1998; Gudelsky et al., 1992; Guinetdinov et al., 1994; Iorio et al., 1983; Kuballa et al., 2005; Magnusson et al., 1987a; Westerink et al., 1984)

Using our simulation model of a striatal varicosity, we determined what changes must occur to cause a sustained elevation in extracellular dopamine and DOPAC. Since inhibition of dopamine D_2 receptors is known to increase dopamine neuron firing rate and dopamine quantal size, we initially used an increase in rate of exocytosis as the only mechanism for increasing extracellular dopamine in our simulation model. However, increased extracellular dopamine levels were not sustained over time. We then asked what additional effects are required to maintain a sustained increase in extracellular dopamine. The only required effect was a small increase in rate of dopamine synthesis for the dopamine signaling pool. The parameter values that produced the desired output were 163% of control for rate of exocytosis and 122% of control for rate of dopamine synthesis in the dopamine signaling pool. The mechanism of production of extracellular DOPAC is not experimentally verified. We have postulated the presence of a DOPAC-synthesis-secretory complex whereby a complex of enzymes synthesizes both dopamine and DOPAC and then exports DOPAC. We used a 90% increase in dopamine synthetic rate in this complex to simulate the antipsychotic drug elicited increase in extracellular DOPAC. After these manipulations, the rate of dopamine synthesis (sum of both pools) was 123% of control, and tissue DOPAC (sum of cytosolic and extracellular DOPAC) was 122% of control [refer to figure 3]. These values for tissue DOPAC and rate of dopamine synthesis were substantially smaller than the experimentally published values.

We next assumed that the antipsychotic drugs exert effects on dopaminergic neurons in addition to antagonism of dopamine D_2 receptors and that these effects influence tissue DOPAC and rate of dopamine synthesis. A search of the literature revealed that some antipsychotic drugs accumulate in isolated synaptic vesicles and inhibit dopamine uptake (Moriyama et al., 1993) and that some antipsychotic drugs are capable of dissipating pH gradient in living mesolimbic neurons (Rayport and Sulzer, 1995). One hypothesis relating to dopamine storage posits that only the neutral form of dopamine is involved in the passive diffusion of dopamine from vesicles to cytosol. A small alkalization of vesicles would increase the fraction of dopamine molecules in the neutral form and increase rate of passive diffusion out from vesicles. Therefore, we used an increase in rate of dopamine passive diffusion from vesicles as an additional effect for the antipsychotic drugs.

To determine whether increases in passive diffusion from vesicles account for the larger increases in tissue DOPAC and rate of dopamine synthesis, we varied values for this parameter in our model. We found that increases in passive diffusion had to be coupled with increases in rate of dopamine synthesis in order to maintain tissue dopamine levels. We found the set of these two values that provided a model output matching experimental data for total tissue DOPAC for each antipsychotic drug. We then plotted the experimentally published values for rate of dopamine synthesis as well as the simulation model value for rate of dopamine synthesis as a function of rate of leak of dopamine out from vesicles (Fig 3). The two indices of dopamine synthesis were very close, suggesting the strategy employed has biological relevance.

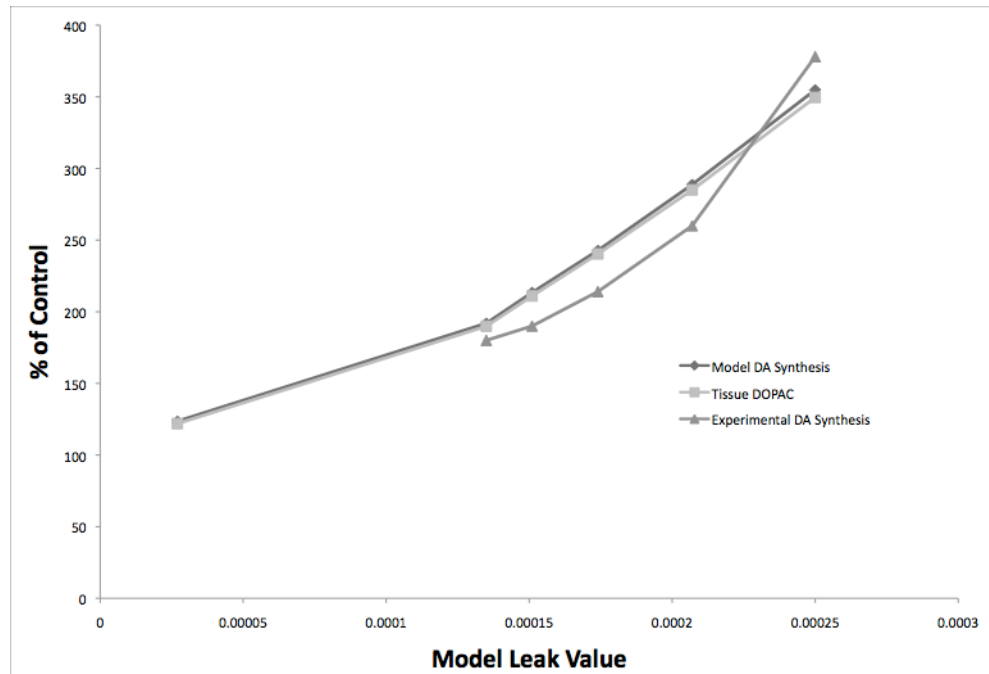


Fig 3. Relationship between rate of dopamine synthesis and rate of passive diffusion needed to maintain constant level of tissue dopamine and produce various increases in tissue DOPAC.

Using the simulation model, pairs of values for rate of dopamine synthesis and for rate of passive diffusion out from vesicles were determined that fulfilled the criteria of providing the increase in tissue DOPAC reported for various antipsychotic drugs and maintaining tissue levels of dopamine at control value. The plot shows rate of dopamine synthesis from both the simulations and from published experimental literature as well as tissue DOPAC levels as a function of rate of passive diffusion out of vesicles. The points associated with the lowest value of leak show the model output associated with drugs increasing extracellular dopamine and DOPAC while having no effect on dopamine leak out from vesicles. For all other points, the simulations included an effect of drugs on rate of passive leak of dopamine out from vesicles, and the model output and experimental data show a close correlation.

Discussion

The major conclusion of this work is that antipsychotic drugs have two principal effects on dopaminergic neurons: antagonism of dopamine D_2 receptors and partial alkalization of neurotransmitter storage vesicles (Fig 4). The effects on dopamine D_2 receptors produce an increase in levels of extracellular

dopamine and extracellular DOPAC along with a small increase in rate of dopamine synthesis. The effect on neurotransmitter storage vesicles causes an increase in rate of passive diffusion of dopamine out of vesicles followed by a compensatory increase in rate of dopamine synthesis in the cytosol, which is required to maintain tissue levels of dopamine. Because more dopamine is passing through the cytosol, more metabolism occurs, and tissue DOPAC levels increase. All antipsychotic drugs evaluated have the same ability to antagonize dopamine D₂ receptors, but the drugs vary greatly in ability to partially alkalinize vesicles.

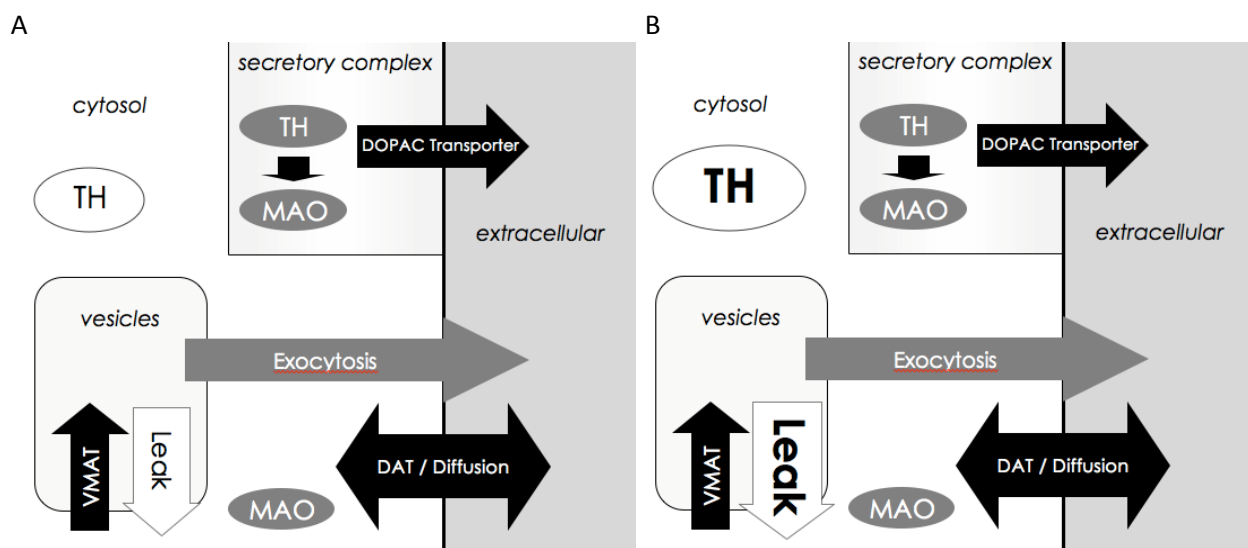


Fig 4. Model for explaining mechanisms occurring with administration of antipsychotic drugs

Antipsychotic drugs act on tyrosine hydroxylase (TH), monoamine oxidase (MAO), exocytosis, and passive diffusion. All antipsychotic drugs increase exocytosis, TH activity in the secretory complex, and MAO activity to a similar extent. Sulpiride (a) has a modest increase on TH activity in the cytosol and passive diffusion of dopamine out of vesicles. Haloperidol (b) has a large increase on TH activity in the cytosol and passive diffusion of dopamine out of vesicles. Font size represents level of activity.

Experimental data suggesting that antipsychotic drugs increase vesicle pH and therefore increase passive diffusion from vesicles include the observation that antipsychotic drugs accumulate in isolated synaptic vesicles and decrease amount of accumulated dopamine (Moriyama et al., 1993). The impact on accumulated dopamine could be explained by either an increase in passive diffusion from vesicles or a decrease in rate of transport of dopamine into vesicles. Our data would support the first of these possibilities. Another paper showed directly that some antipsychotic drugs are capable of dissipating pH gradient in living mesolimbic neurons (Rayport and Sulzer, 1995). This study also documented that haloperidol was most potent, clozapine intermediately potent, and sulpiride least potent at inducing this effect, an order of potency comparable to that for increasing rate of dopamine synthesis and levels of tissue DOPAC. In a study evaluating effects of amphetamine on dopamine storage in vesicles (Wallace and Connell, 2008), the impact of changing pH on rate of passive diffusion of dopamine out of vesicles was modeled. Using that model, we estimate that the pH of vesicles in the presence of antipsychotic drugs ranges from 5.75 for sulpiride to 6.00 for haloperidol as compared to 5.50 in the absence of drugs. This suggests that only small changes in vesicle pH are needed to produce the observed effects.

Before the advent of microdialysis technique, tissue DOPAC was used as a surrogate estimate of amount of signaling dopamine. Since DOPAC production occurs only by action of monoamine oxidase on dopamine molecules in the cytosol, changes in tissue DOPAC should reflect changes in levels of cytosolic dopamine. Currently, we believe there are three sources of cytosolic dopamine: newly synthesized dopamine, signaling dopamine in transit between recapture from extracellular compartment and storage in vesicles, and dopamine passively diffusing out from vesicles. If rate of dopamine synthesis and rate of passive diffusion of dopamine remain constant, changes in tissue DOPAC will provide a reliable estimate of the signaling pool of dopamine. However, our data suggest the diffusion-restorage pool is not constant in the presence of antipsychotic drugs. Thus, tissue levels of DOPAC after administration of antipsychotic drugs do not accurately reflect the changes in the signaling pool of dopamine.

Haloperidol appears to be by far the most studied antipsychotic drug in various animal models. One of the themes from haloperidol studies is that antipsychotic drugs increase the amount of dopamine involved in signaling – an effect inferred from the large increases in tissue DOPAC elicited by this drug. As demonstrated in this paper, haloperidol induces substantially larger increases in tissue DOPAC than do most antipsychotic drugs and tissue DOPAC is not always a good index of amount of signaling dopamine. Our data suggest that antipsychotic drugs induce only a small (~66%) increase in signaling dopamine. As dopamine D₂ receptors are blocked by the antipsychotic drugs, this small increase in signaling dopamine would only act on dopamine D₁ receptors.

One conclusion from our model is that an increase in rate of dopamine synthesis in the cytosolic compartment is required to maintain tissue dopamine levels in the presence of an increased rate of passive diffusion of dopamine out from vesicles. This conclusion suggests that a sensor monitoring vesicular levels of dopamine and linked to control of tyrosine hydroxylase activity must be present. Such a regulatory system has not yet been experimentally verified. Other data also suggest that additional regulatory mechanisms for tyrosine hydroxylase must exist. For example, both cytosolic and extracellular dopamine increase in the presence of amphetamine (Wallace, 2012), and both of these effects are known to decrease rate of dopamine synthesis (Dunkley et al., 2004; Lindgren et al., 2003; Lindgren et al., 2001; Nagatsu et al., 1964). However, the experimentally measured effect is that rate of dopamine synthesis is increased in the presence of amphetamine (Carenzi et al., 1975; Costa et al., 1972; Demarest et al., 1983; Elverfors and Nissbrandt, 1992; Kehr et al., 1977; Kuczenski, 1977; Pearl and Seiden, 1979). One potential hypothesis related to findings in the current work is that tyrosine hydroxylase and aromatic amino acid decarboxylase enzymes might be associated with dopamine storage vesicles. Early biochemical studies of striatal homogenates identified a fraction of tyrosine hydroxylase activity existing in membranous fractions (Kuczenski and Mandell, 1972). Tyrosine hydroxylase also contains several phosphorylation sites (Dunkley et al., 2004), opening the possibility that post-translational modifications of the protein might determine location within a varicosity. Variants in post-translational modifications might specify one population of enzymes to storage vesicles and another to the plasma membrane to participate in the DOPAC synthesis-secretory complex used in our model.

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